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Original Research

Heritable variations of *Pteris biaurita* L. discovered by ISSR markers in the Western Ghats of Tamil Nadu, India

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ABSTRACT:

Inter Simple Sequence Repeats (ISSR) markers were utilized to identify the levels of heritable varieties and patterns of the populace structure among the five populaces of *Pteris biaurita*, a natural fern in India. A comprehensive examination was directed in three replicates at 2013-14 seasons in the Western Ghats, South India. Five wild *P. biaurita*, accessions (maiden hair) were assessed for genotyping studies. Results demonstrated a pivotal discrepancy among genotypes for they were characterized in view of this uniqueness in four groups by the genetic cluster examination. In this trial, ISSR primers amplified 63 polymorphic groups. In view of the genetic identity data, genotypes were figured and differed from 0.5714 to 0.6984. The percentage of polymorphism indicated predominant genotype that may be utilized for the conservation of species. ISSR appeared to be an obliging marker for prediction of genotype inside a closed group of inter specific populace in the investigation territory.

Keywords:

ISSR analysis, *Pteris biaurita* L, genetic variation, Western Ghats

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INTRODUCTION

The Indian subcontinent is provided with a mind blowing cluster of medicinal plants which involve the main reserve base of the social framework in the nation. The affluent bio-diversity is additionally blended with similarly rich health care system. It's rich vegetation and diversity is absolutely because of the enormous assortment of the atmosphere and altitudinal varieties tied with the different biological environments.

The Western Ghats of peninsular India is of terrific phyto-geographical consequence which constitutes one of the 34 worldwide biodiversity hotspots alongside Sri Lanka, on clarification of extraordinary levels of plant endemism and more elevated amounts of territory loss. Fern in Western Ghats of South India, south of Palghat gap include around 33% of the fern flora of India. The greater part of them dwell on streams and stream banks in evergreen timberlands and shores past 800m while some reside on roadsides and clearings. (Manickam, 1995).

Regardless of its moderately remarkable rate, distance colonization is of unbalanced significance to species range extensions. This incorporates diaspore attributes, plant ontogenetic and morphological characteristics and also reproductive systems. Genotypes having these capacities will have a fussy increase over different genotypes while colonizing new and remote territories. This basic aspect is getting more attention in a world progressively under the factor of environmental change and fracture of natural living spaces.

Different examinations on plants and animals have demonstrated that people with higher spreading limits tend to be found with more prominent occurrence towards species' range limits and that these enhanced limits have a tendency to have a hereditary basis. In like manner, inbreeding rates frequently increment towards a progression of margins.

Dimension of genetic diversity are vital for considering preservation of a specific spaces. A decrease in hereditary uniqueness can destabilize the capacity of a animals to react to the natural selection and thus confines its evolutionary budding. Petite populaces are, a ton, subjected to the loss of alleles through genetic drift, or arbitrary changes in the allele frequency. Along these lines any reexamine in genetic diversity of *Pteris biaurita* needs to address the above issues.

DNA markers have demonstrated valuable in crop breeding, particularly in the examinations on hereditary differences and gene mapping. ISSR (Inter Simple Sequence Repeats) is such a DNA based marker technique which could be utilized for demonstrating genetic changeability. Changes in DNA sequences and single base sub-situations including DNA conformation changes can be distinguished as movements in electrophoretic mobility utilizing these strategies.

In this present examination, *Pteris biaurita* L, were taken from Western Ghats of Tamil Nadu. *Pteris biaurita* L, is found in the fields and lower inclines of the slopes of Punjab, Rajasthan, West Bengal, Tamil Nadu and Maharashtra. This fern yields isoadiantone, fernene, hentriacontane, hentriacontanone-16 and beta-sitosterol on different extraction utilizing phytochemical methods. The plant concentrate of *Pteris biaurita* L. (Adiantaceae) is additionally utilized as a part of the treatment of cough, diabetes, and skin ailments (Manickam, 1995).

The perplexity of this species, standard at its species level is an extraordinary hazard to the Pteridologists who attempt to spot it for its valuable uses. The morphology stands comparable with that of the barely related species aside from minute peculiarity with its chromosomal nature and chemistry stands peculiar. Accordingly conspiring an exact arrangement for its species ID in spite of its variety remains as a massive errand certainly. Additionally, the predominant genotype of the species was perceived so that the conservation of the species made simple, if unique activities are taken sooner rather than later.

Table 1. Place of collection of the plants and their accession ID

S. No.	Species	Accession ID	Location
1.	*Pteris biaurita*	POP 1	Kothayar
		POP 2	Gundar
		POP 3	Thirugurankudi
		POP 4	Kodaikanal
		POP 5	Kadana dam

Table 2. ISSR primers and their sequences

S. No.	Name of the primer	Primer Sequences
1	B07	$(CG)_5AG$
2	B09	$(ATG)_3CA$
3	L03	$(GC)_4$ AT
4	A12	$(GA)_6CC$
5	A13	$(GT)_6CC$

MATERIALS AND METHODS

Study area

Five Western Ghats regions *viz.,* Kothayar, Gundar, Thirugarankudi, Kodaikanal and Kadana Dam are chosen for the study due to the ease of use of this three species unvaryingly (Table 1). Ten ISSR markers were veteran which showed steady amplification in five markers at five sampling sites. This has provided a clear idea about the genetic diversity of *Pteris biaurita* in its species level.

DNA isolation

Pteris biaurita, individuals were collected from the Western Ghats of Tamil Nadu, India. DNA was extracted from youthful leaves using the method described by Dellaporta *et al.* (1983). The DNA isolated was purified using Phenol- Chloroform method and the purity of the DNA samples were determined using UV-Spectrophotometer at the optical density of 260 nm and 280 nm; the DNA samples were diluted to 25 ng μl^{-1} for PCR amplification.

ISSR amplification

ISSR amplification reactions were carried out in 25-µl volume containing 50 ng template DNA, 0.5 U Taq DNA polymerase, 10 mM dNTP, 10 µM primer in 1× reaction buffer that contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, and 0.01% gelatine (Williams *et al.,* 1990). Amplifications were performed in an Eppendorf Master Cycler gradient. Amplification conditions were one cycle at 94°C for 4 min, and 94°C for 30 s, 55°C for 45 s, followed by stepwise reduction of 1°C for the first five cycles, and 72°C for 2 min. In subsequent 35 cycles, annealing temperature was maintained at 50°C, followed by one cycle of 7 min at 72°C. Amplified yields were loaded on 2% agarose gel and separated in 1× TBE buffer at 75 V. The gels were visualized under UV after staining with ethidium bromide and documented using a gel documentation and image analysis system. The primers used for the ISSR analysis are listed out in Table 2.

Data Analysis

The gels from ISSR investigation were visualized at gel documentation system (Alpha Imager 1200). Based on the primary data (presence or absence of bands), pair wise genetic distance between the samples was calculated using NTSYS and POPGENE packages.

Table 3. ISSR profile of *Pteris biaurita* using selected primers

S. No.	ISSR Primers	Total Number of Bands	Total Number of Polymorphic bands	% Polymorphism
1.	B09	63	13	20.63
2.	B07	63	17	26.98
3.	A21	63	15	23.80
4.	L03	63	12	19.04
5.	A13	63	06	09.52
		Total		99.97

Table 4. Nei's original measures of genetic identity and genetic distance in *Pteris biaurita*

POP ID	1	2	3	4	5
1	*****	0.6667	0.6349	0.6825	0.6190
2	0.4055	*****	0.6190	0.6984	0.5714
3	0.4543	0.4796	*****	0.6984	0.6984
4	0.3819	0.3589	0.3589	*****	0.6508
5	0.4796	0.5596	0.3589	0.4296	*****

Table 5 Over all genetic variation statistics for all loci in *Pteris biaurita* L.

Sl. No.	Parameters	Values
1.	Observed numbers of alleles	1.6667
2.	Effective numbers of alleles	1.4933
3.	Nei's (1973) gene diversity	0.2768
4.	Shannon's Information Index (Lewontin, 1972)	0.4021
5.	Overall percentage of polymorphism	66.67

Table 6. Distance between and population length in *Pteris biaurita*

Between	And	Length
4	3	3.32772
3	Pop1	19.68499
3	1	1.73774
1	Pop2	17.94725
1	Pop4	17.94725
4	2	5.06565
2	Pop3	17.94725
2	Pop5	17.94725

RESULTS AND DISCUSSION

Examination of five assessment of *Pteris biaurita* open 63 polymorphic loci on trialing with five potential ISSR primers. Ten primers were analyzed of which five primers (Table 4). created reproducible, informative and effortlessly scorable ISSR profiles. An aggregate of 315 bands were scored out, rayed from 6 to 17 per prime (Table 4). The genetic distance flanked by the populace extended from 0.3589 to 0.4796 and the genetic identify varied from 0.5714 to 0.6984 (Table 6). The overall observed and effective numbers of alleles were 1.6667 and 1.4933 respectively and overall genetic diversity was 0.2768 (Table 1; Figure 2). The Shannan's information index was observed to be 0.4021. The overall percentage of polymorphism was 66.67.

The number of polymorphic loci and percentage of polymorphism was calculated by using the software POPGENE package version 1.3.2. Among these five populations, populations 1, 2 and 3 (Kothayar, Gundar and Thirugurankudi) showed high polymorphism. Considering these three populations, population 2 (Gundar) showed highest polymorphism (Figure 1).

Hence, among the five accessions of *Pteris biaurita* in the Gundar accession (Pop 2) is well thought -out as superior genotype, due to the high percentage of polymorphism in ISSR analysis (Table 6).

Dendrogram was drawn based on Nei's (1972) with the consideration of genetic distance. The methodology followed is UPGMA based on neighbor joining method of PHYLIP Version 3.5.

The dendrogram of *Pteris biaurita* L. (Figure 1) produced two clusters. Cluster 1 is superior to cluster 2 containing populace 1, 2 and 4. Here populace 2 and 4 are more personally related to each other than populace 1. In the cluster 2, populace 3 and 5 forms a separate clade. It is understood that there is considerable amount of genetic variability between the populations 3, 5 and 1, 2, 4 of *Pteris biaurita* L. (Figure 2).

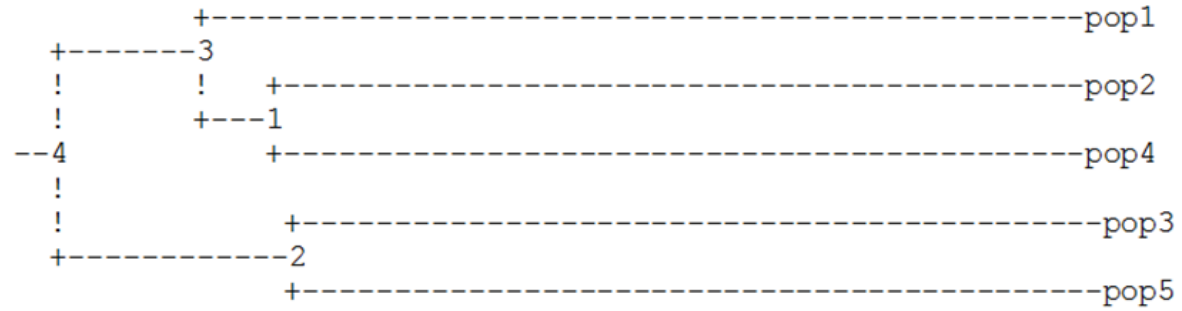

Figure 1. UPGMA dendrogram of *Pteris biaurita* based on Nei's genetic distance derived using ISSR markers

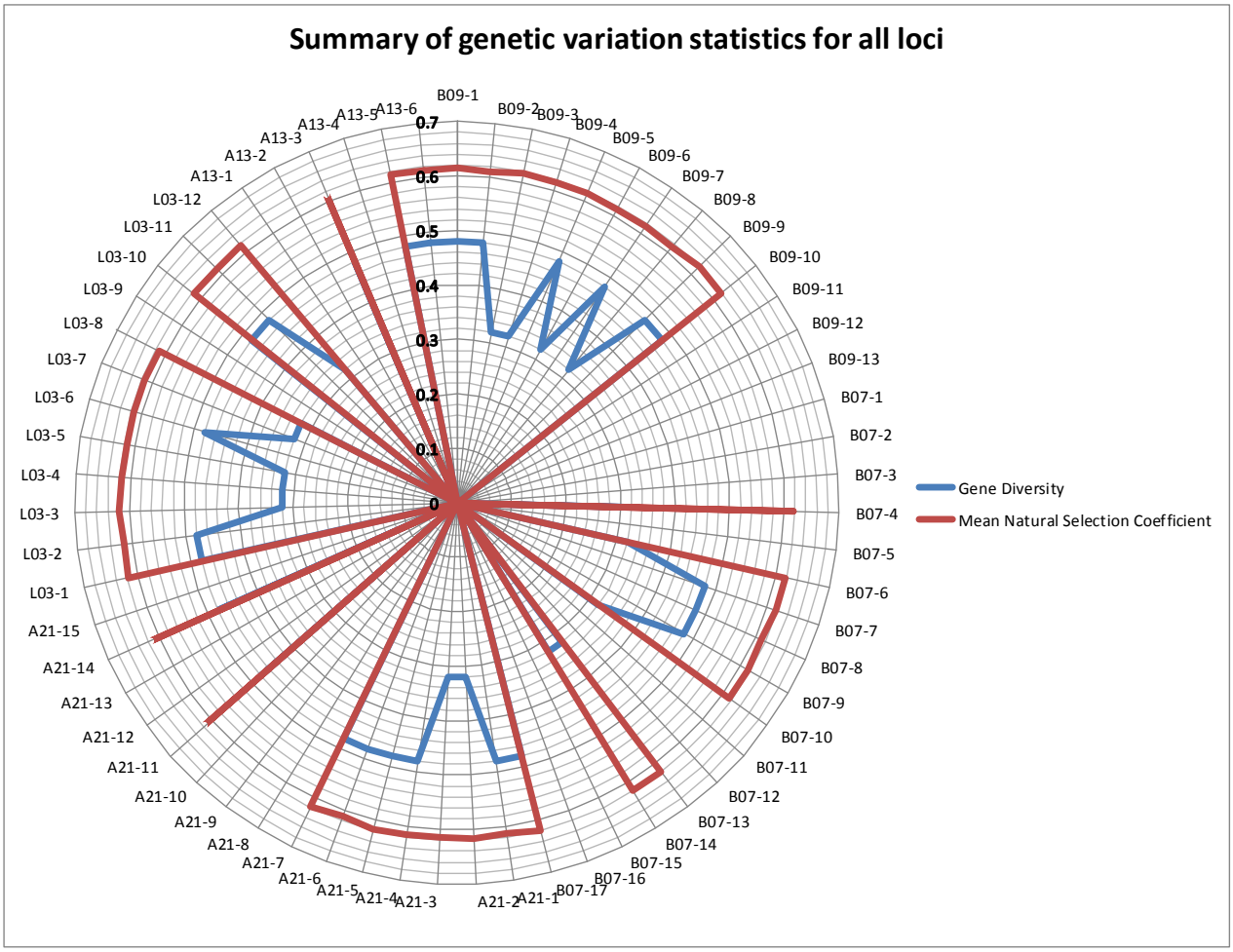

Figure 2. Summary of genetic variation statistics for all loci in *Pteris biaurita*

The morphological varieties were counter affirmed by the genetic differences in the plants through ISSR markers. Particular primers that arbitrate between the species were perceived effectively. The hereditary relationship exemplified by the molecular markers by means of DNA fingerprinting demonstrates their nearness and relativity. Out of ten primers five uncovered steady banding pattern and in this way uncovered variability inside the species.

ISSR markers were utilized to analyze genetic demarcation inside and between the chose species. Investigation of genetic variability showed all the five populaces analyzed, sexual recombination had been the greatest establishment of hereditary variety than asexual proliferation (Mes, 1998; Hulst 2000; Kjolner *et al.,* 2006). This decision is in congruity with different past examinations, which have almost utilized allozyme markers to derive the mating frameworks working in normal populaces of the related and locally accessible ferns.

Allozyme analysis by Soltis and Soltis (1987) and Ranker and Geiger (2008) demonstrated that populaces of diploid homosporous ferns are generally ruled by sexual random mating or outcrossing. Besides, gene flow from the adjoining populaces would moderate the broadening yet just by means of sexual proliferation. Associated designs have been recognized in various other ferns (Ranker, 1992; Ranker and Geiger, 2008; Rumsey *et al.,* 1999; Chen *et al.,* 2010).

The purpose behind this hereditary variety is probably emerged from contrasts in the DNA content of the progenitor species. This proposal is reliable with the

event of diploid apomicts in different plants, including taxa of the *Pteris biaurita* (Pravin, 2005). The clades are developed through Popgene 2.1 and related ancestries indicate brawny patterns of reticulate fruition. Higher the genetic diversity of these species, more prominent is their practicality in the earth.

Researches have brought up that this induction ought to be confined to close relatives, and the distinction amongst diploids and their autoploid offspring (Moran, 1982; Barrington *et al.,* 1986). Our discoveries are predictable with proposals that the high chromosome numbers, and conserved chromosome sizes revealed for some homosporous ferns. This has been recommended to be because of a higher degree of consistency of chromosomes and the possible concealment of transposable components (TEs) in homosporous ferns (Barker, 2013; Bainard *et al.,* 2011).

It likewise affirms with the theory that genomes size variation in homosporous ferns are driven by poly-ploidisation. Our examination proves that the genome evolution is happening in these plants; without a doubt, given the degree of hybridization and are most likely more broad across ferns, however, may have been to a great extent disregarded because of the reticulate advancement detailed in homosporous ferns; in general, it appears to be likely that adjustments in genome size low level of sampling.

CONCLUSION

Genetic diversity between *Pteris biaurita* species found on the Western Ghats region is identified. ISSR markers proved amplification in the selected species, thus validates its genetic variation strategy

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